A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(dopamine/homovanillic acid/5-hydroxyindoleacetic acid/3-methoxy-4-hydroxyphenylethylene glycol)

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ABSTRACT A syndrome similar to idiopathic parkinsonism developed after intravenous self-administration of an illicit drug preparation in which N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP) might have been responsible for the toxicity. In the present study we show that intravenous administration of NMPTP to the rhesus monkey produces a disorder like parkinsonism (akinesia, rigidity, postural tremor, flexed posture, eyelid closure, drooling) that is reversed by the administration of L-dopa. NMPTP treatment decreases the release of dopamine and dopamine accumulates in swollen, distorted axons in the nigrostriatal pathway just above the substantia nigra, followed by severe nerve cell loss in the pars compacta of the substantia nigra and a marked reduction in the dopamine content of the striatum. The pathological and biochemical changes produced by NMPTP are similar to the well-established changes in patients with parkinsonism. Thus, the NMPTP-treated monkey provides a model that can be used to examine mechanisms and explore therapies of parkinsonism.

The most prominent pathological change in idiopathic parkinsonism is degeneration of the nigrostriatal dopaminergic pathway with nerve cell loss in the substantia nigra (1). A neurochemical consequence of this loss of dopaminergic neurons is a marked decrease in the concentrations of dopamine and its major metabolite homovanilllic acid (HVA) in the caudate nucleus and putamen (2). The effectiveness of L-dopa and direct-acting dopamine agonists in reversing akinesia, rigidity, resting tremor, and postural abnormalities in patients with idiopathic parkinsonism (3, 4) reflects the pathophysiology of these clinical signs.

In 1979 a single case of parkinsonism occurring after intravenous self-administration of an illicit narcotic analgesic was described (5). Recently, a series of similar cases has been reported (6). We had the opportunity to examine two patients included in the later study. In both instances there was evidence that the method of Ziering et al. (7) had been used to synthesize the reverse ester of meperidine, 4-propionoxy-4-phenyl-N-methylpiperidine. The injected mixture also contained the side product N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP). After intravenous administration of multiple doses of the drug mixture over several days, the patients gradually developed persistent symptoms of parkinsonism, with a syndrome characterized by severe akinesia, rigidity, a flexed posture, and a resting tremor associated with low concentrations of HVA in the lumbar cerebrospinal fluid (5–6.7 ng/ml). The clinical signs were reversed by the administration of L-dopa or bromocriptine. A marked loss of pigmented cells in the substantia nigra with minimal changes in the locus ceruleus was found in the one patient who died of other causes 18 months after the onset of symptoms (5).

In the present study we show that repeated intravenous administration of NMPTP to the rhesus monkey over a period of 5-8 days produces a chronic disorder with neurological, biochemical, pathological, and pharmacological similarities to idiopathic parkinsonism. The symptoms were suppressed by the administration of L-dopa.

METHODS

Subjects and Sample Collection Procedures. Twelve male or female adult rhesus monkeys (Macaca mulatta) weighing 5–8 kg were used in these studies; eight animals were given NMPTP and four animals served as controls. The monkeys were adapted to primate restraining chairs and kept in heated and humidified quarters on a 12-hr light/dark cycle (light 0700–1900). They were fed Purina Monkey Chow once daily and given water ad lib.

In two animals, stainless steel cannulae were placed in the lateral ventricle near the foramen of Monro. A 3-m length of polyethylene tubing (inside diameter, 0.58 mm; vol, 0.79 ml) was used to connect the cannulae to a fraction collector housed in a refrigerator (4°C). A timing circuit and a solenoid valve allowed intermittent flow (20–40 sec open, 360–500 sec closed) and a controlled collection rate (1.25–1.5 ml/hr). The cerebrospinal fluid (CSF) within the tubing was at room temperature for approximately 30 min before entering the refrigerated compartment. The collected CSF was frozen (−20°C) until assayed for biogenic amine metabolites. The cannula was replaced by a stylet and a protective cap when the animal was returned to its cage between collection periods.

Drug Administration. Crystalline NMPTP (Aldrich, 97%) was dissolved in ethanol (8.7 mg/ml) and diluted with 9 vol of water. Immediately before injection, this solution was sterilized by filtration through a 22-μm-pore filter.

NMPTP was administered intravenously in 1-2 min to eight animals with various dosage schedules. Monkey 1 initially received three doses of 0.15 mg/kg at 24-hr intervals. After 72 hr, this animal was given three additional daily doses of 0.30 mg/kg, for a total of 9.9 mg during 8 days. Monkey 2 was given four daily doses of 0.30 mg/kg followed by three daily doses of

Abbreviations: NMPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; HVA, homovanilllic acid; 5-HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; CSF, cerebrospinal fluid.
0.38 mg/kg on consecutive days for a total of 17.1 mg during 7 days. Monkey 3 received four daily doses of 0.35 mg/kg followed by one dose of 0.40 mg/kg and one of 0.69 mg/kg for a total of 18.6 mg during 5 days. Monkeys 4–8 received five doses of 0.33–0.36 mg/kg for a total of 11.0 mg during 5 days.

**Brain Removal and Dissection.** Animals were injected intramuscularly with ketamine (Vetalar) at 7 mg/kg and xylazine (Rompun) at 0.6 mg/kg and then anesthetized with 50 mg of thiamylal (Siril) given intravenously, and the trachea was intubated. A mixture of air and O₂ was used to ventilate the animal, and 120 mg of pentobarbital (Somni) was administered intravenously to deepen the anesthesia. After the cranium was removed and the dura over the hemispheres was exposed, a lethal dose of pentobarbital (360 mg) was administered. The brain was transected at the level of the foramen magnum, removed, and placed on a metal dissection tray on ice within 5 min of the cessation of respiration. This procedure required 20–25 min to complete from the time of ketamine administration. The brain was bisected in the sagittal plane and the left half was used for dissection of brain regions based upon gross anatomical landmarks. After dissection, brain tissue samples were weighed, frozen on dry ice within 20 min, and stored in a −70°C freezer until assayed. Tissue samples of specific brain nuclei (putamen, caudate) were obtained from slices of the right half by punch microdissection and immediately frozen on dry ice.

**Biochemical Methods.** Tissues were obtained for analysis of HVA, 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), and dopamine. Brain tissue samples were homogenized in 5 ml of 0.2% ascorbic acid/0.02% EDTA solution containing [³H]HVA at 100 ng/ml, [³H]5-HIAA (Merck Sharp & Dohme) at 100 ng/ml, and [³H]-MHPG (8) at 50 ng/ml as internal standards. After centrifugation at 10,000 × g, an aliquot (2 ml) of the supernatant was extracted twice with 7 ml of hexane and then used for the simultaneous determination of HVA and 5-HIAA. The aqueous phase was acidified by addition of 0.5 ml of 6 M HCl and extracted with 7 ml of ethyl acetate. The organic extract was evaporated to dryness, and the residue was redissolved in 1 ml of ethyl acetate that had been dried with anhydrous Na₂SO₄.

The ethyl acetate was evaporated under a stream of N₂ and the residue was dissolved in 150 μl of 1:2 mixture of trifluoroethanol and trifluoroacetic acid anhydride (Pierce) and heated at 70°C for 15 min. The mixture was evaporated and the residue was dissolved in 200 μl of trifluoroacetic acid anhydride and heated at 70°C for 60 min. After evaporation, the residue was taken up into 50 μl of dried ethyl acetate and analyzed by gas chromatography/mass spectrometry using a Finnigan 3200 quadrupole mass spectrometer. The ion pairs monitored were at mass-to-charge ratio m/z 360 and 365 for endogenous and deuterated HVA and at 465 and 467 for endogenous and deuterated 5-HIAA, respectively. The peak height ratios were determined and the amounts of endogenous HVA and 5-HIAA were calculated by inverse linear regression analysis of standard curves. Peak heights were corrected for channel spillover and natural abundance of deuterium by using the program of Jenden et al. (9). Separation was carried out with a 1.5 m × 2 mm inside diameter glass column packed with 3% OV17 on 100–120 mesh Gas Chrom Q (Applied Science, State College, PA). The column was maintained at 155°C for HVA and 180°C for 5-HIAA, with retention times of 1–1.5 min. A second aliquot (2 ml) of the supernatant was used for the determination of MHPG by a modification of the method of Gordon et al. (10).

The concentrations of dopamine and HVA in the brain tissue samples obtained by the punch technique of Palkovits (11) were determined by HPLC with electrochemical detection (12) and indexed to the protein content determined by the Lowry method (13). For histofluorescence studies, the brain, including the brainstem, was cut in the coronal plane into 5- to 10-mm-thick slices, which were placed on glass slides and immediately frozen on dry ice. The frozen slices were sectioned at 20 μm with a cryostat (−18°C). Tissue slices were stained for catecholamines by utilizing the glyoxylic acid method of histofluorescence (14).

The concentrations of HVA and 5-HIAA in CSF were determined by gas chromatography/mass spectrometry as outlined above. After the addition of 500 ng of [³H]HVA and 125 ng of [³H]5-HIAA, 1 ml of CSF was diluted with 2 ml of 0.2% ascorbic acid and acidified with 0.3 ml of 3 M HCl, and 50 μl of 0.2% EDTA was added. The aqueous mixture was extracted with ethyl acetate and the extracted metabolites were carried through the steps outlined above. The MHPG concentration in CSF was determined by the method of Jimerson et al. (15).

**Therapy with L-Dopa.** Monkey 2 received oral Sinemet for a 2-month period. One tablet of Sinemet (100 mg of L-dopa and 10 mg of carbidopa) was pulverized, dissolved in orange drink, and given to the animal every 4 hr five times daily. During this treatment motor activity was continuously recorded by using an acceleration-sensitive device equipped with a solid-state memory that stored data on the number of movements per unit of time (7.5 min) for a period of up to 32 hr (16). The activity monitor was placed in the midline back pocket of a primate jacket.

**Histopathology.** For neuropathological studies, monkey 2 was killed 2 months after the last dose of NMPTP. The brain was fixed in 15% formalin and tissue sections were stained with hematoxylin and eosin.

**RESULTS**

**Behavioral Observations.** The acute effects of NMPTP included abnormal movements and alterations of motor behavior and posture. These effects were first seen after two or three doses of NMPTP (0.33 mg/kg) had been administered at 24-hr intervals. The abnormalities became more striking after each successive dose. They occurred within 5 min of drug administration and, initially, lasted for 15–30 min. After an animal had received four or five doses, some of the acute motor effects persisted.

The first motor signs to appear, usually after the third dose, were intermittent eyelid closure, a decrease in spontaneous movements, including loss of facial expression, and postural tremor. The animals were awake, however, and responded to loud noises by opening their eyes, looking at the examiner and making weak threatening movements. The tremor was intermittently present, moderate in amplitude, and slow in frequency, and involved the proximal muscles of the extremities. A postural tremor of the head or jaw was observed in some animals. These acute motor effects lasted up to 30 min.

Motor signs that appeared only after four or five doses included abnormal facial movements and changes in posture, muscle tone, and deglutition. Twitching of the facial muscles and facial grimacing were prominent effects seen in all of the animals. Extension of the head, rigidity of the upper and lower extremities demonstrated by passive range-of-motion testing, and sustained turning to one side were observed in some of the animals. Some animals also had difficulty swallowing, as evidenced by drooling and the accumulation of food biscuits in their mouth pouches. Rotary movements of the eyes were observed in some animals.

In all of the animals eyelid closure, decreased spontaneous motor activity, rigidity, postural tremor, and difficulty swallowing persisted after a cumulative dose of about 1.7 mg/kg had been reached. Abnormal facial movements, head extension, and rotary eye movements, however, were observed only during the 30 min immediately after drug administration. After the 5-day period of drug administration, other signs of
motor impairment appeared. These included general slowness of movement, a flexed posture, loss of hand dexterity, and "freezing" episodes. The animals remained seated, with marked flexion of the neck, thoracic spine, and upper and lower extremities (Fig. 1 Upper). There was evident difficulty in picking up food biscuits, which were subsequently dropped while being carried to the animal's mouth. Episodes of "freezing" or stopping in the middle of a motion were observed in some animals. "Kinesie paradoxale" (17) was apparent in some animals; when a biscuit was thrown into the bottom of the cage, the animals initially moved with normal speed to retrieve the food but then their movements became slow again. Motor function deficits appeared to increase during the initial 2-week period after the last dose of the drug. Treatment with L-dopa was effective in correcting the motor deficits. During the period 30 min to 3 hr after the oral administration of Sinemet, motor activity returned to normal or became abnormally high (Figs. 1 Lower and 2).

**Neurochemical Changes.** The changes in the concentrations of HVA, MHPG, and 5-HIAA in ventricular CSF during multiple dose administration were similar in the two animals studied, although the initial (0.15 and 0.30 mg/kg) and total (9.9 and 17.1 mg) doses differed (Fig. 3). After the period of drug administration (at 6 and 8 days), the concentrations of HVA, MHPG, and 5-HIAA had fallen to an average of 37, 38, and 60% of the base line value, respectively. The greatest decrease in the CSF concentrations of HVA and MHPG occurred during the first day, when the levels fell rapidly to an average of 61% and 52%, respectively, of the base line values. The concentration of 5-HIAA decreased gradually during multiple dose administration.

The levels of HVA, MHPG, and 5-HIAA in various brain regions were generally reduced at 1 to 5 days after the administration of five doses of NMPTP totaling 11 mg (Fig. 4). The levels of HVA were uniformly decreased to an average of 37% of the control values; the midbrain, putamen, and caudate nucleus contained 36%, 32%, and 31% of control HVA concentrations, respectively. MHPG levels were decreased in all brain regions examined to an average of 33% of the control values except in the frontal cortex. Levels of 5-HIAA were also decreased in the midbrain, putamen, and caudate nucleus, as well as in the thalamus, caudate nucleus, and putamen (Fig. 5). The decrease in 5-HIAA levels was more pronounced in the thalamus and putamen than in the caudate nucleus.

**Fig. 1.** (Upper) Flexed posture associated with akinesia exhibited by NMPTP-treated monkey 2 16 hr after last dose of L-dopa. (Lower) Reversal of abnormal posture and akinesia 1 5 hr after treatment with oral Sinemet (100 mg of L-dopa and 10 mg of carbidopa) to monkey 2. This photograph was taken 2 hr after that shown in Upper.

**Fig. 2.** Number of movements per 30 min recorded for a 24-hr period by an acceleration-sensitive activity monitor placed in the pocket of a primate jacket. Motor activity level of NMPTP-treated monkey 2 while receiving L-dopa (Upper) compared with that of a normal male monkey (Lower). Sinemet (100 mg of L-dopa and 10 mg of carbidopa) was administered orally at times indicated (D).
as the hypothalamus, but the 5-HIAA in the medulla, pons, and frontal cortex did not appear to be altered significantly.

In the one animal that was restudied after 3 months, the HVA concentration in the ventricular CSF remained as low as the lowest levels found initially, whereas the concentrations of MHPG and 5-HIAA had increased (Fig. 3). The MHPG concentration, which had been as low as 37% of its base line value, returned to 75% of this value 3 months later and the 5-HIAA concentration increased from 66% to 86% of its base line value.

The dopamine levels of the putamen were about twice normal in two animals killed 1 day after the scheduled (11.0 mg of NMPTP during 5 days) drug administration (Table 1). The molar ratio of HVA to dopamine in these animals was decreased

![Figure 3](image1.png)

**FIG. 3.** Ventricular CSF concentrations of 5-HIAA (○), HVA (●), and MHPG (▲) vs. time during the period of repeated intravenous administration of NMPTP (arrows) and 3 months later in NMPTP-treated monkey 1. Zero represents time of administration of the first dose; initial data points were at 2, 6, 10, 14, 18, and 22 hr. Base line values (100%), representing the mean of the concentration values during the 3-day base line period, for 5-HIAA, HVA, and MHPG are 131, 810, and 22.8 ng/ml, respectively.

![Figure 4](image2.png)

**FIG. 4.** Effect of repeated intravenous administration of NMPTP (five doses of 2.2 mg at 24-hr intervals) on the tissue levels of HVA, MHPG, and 5-HIAA in different brain regions of monkeys sacrificed 1–5 days after drug administration. Each bar or line (100% level) represents the mean ± SEM of three animals except bars for hypothalamus, putamen (MHPG only), and frontal cortex, and line for pons (5-HIAA only), which represent two animals. The control values (100%) for the medulla, pons, midbrain, hypothalamus, putamen, caudate nucleus, and frontal cortex, in that order, are as follows: (i) HVA (μg/g), 0.514, 0.962, 3.14, 2.20, 16.3, 12.5, and 0.387; (ii) MHPG (μg/g) 117, 184, 195, 815, 109, 165, and 144; (iii) 5-HIAA (μg/g) 0.963, 1.56, 2.55, 0.963, 0.870, 0.457, and 0.107.

<table>
<thead>
<tr>
<th>Time of sacrifice, days,*</th>
<th>Animal</th>
<th>Putamen Dopamine</th>
<th>Caudate Dopamine</th>
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</thead>
<tbody>
<tr>
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<td>147</td>
<td>153</td>
</tr>
<tr>
<td></td>
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<tr>
<td>27</td>
<td>1</td>
<td>3.2</td>
<td>29</td>
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</tbody>
</table>

All animals received five doses of 2.2 mg of NMPTP intravenously at 24-hr intervals.

* Days after completion of course of treatment with NMPTP.

In one animal studied at 27 days after the 5-day course of drug administration, the dopamine levels of the putamen and caudate nucleus were less than 10% of the control values (Table 1). The HVA to dopamine ratio was increased in the putamen (from 1.4 to 7.6) and the caudate nucleus (from 0.8 to 5.4) at 27 days.

![Figure 5](image3.png)

**FIG. 5.** Transverse sections through the midbrains showing the substantia nigra of a normal monkey (Upper) and NMPTP-treated monkey 2 (Lower). Note severe nerve cell loss in NMPTP-treated animal. (Hematoxylin/eosin stain; ×90.)
Examination of Brain by Histofluorescence. Histofluorescence studies were carried out in one control animal and in animals killed 1 day and 27 days after the 5-day course of drug administration. All of the fluorescent cell bodies normally observed in the substantia nigra had disappeared 1 day after the course of treatment. However, at 27 days approximately half of the normal number of cells were present. At this time swollen, distorted, intensely fluorescent dopamine-containing axons were seen in the area immediately above the zona compacta of the substantia nigra. Swollen axons were also seen in the ventral part of the internal capsule, the basal portion of the globus pallidus, and the adjacent medial part of the putamen. These changes were present at 1 day, but were much more striking in the animal killed 27 days after drug administration. The nucleus accumbens, olfactory tubercle, locus ceruleus, and paraventricular and other hypothalamic nuclei of both drug-treated animals (killed at 1 day or 27 days after NMPTP) appeared normal.

Histopathological Changes. Severe nerve cell loss in the pars compacta of the substantia nigra of monkey 2 (killed 2 months after NMPTP treatment) was found on light microscopy; less than 10% of the normal cell population was present (Fig. 5). In the striatum, no loss or degeneration of nerve cells or reactive glial cell changes were seen.

**DISCUSSION**

NMPTP-induced parkinsonism in man and idiopathic parkinsonism exhibit the same clinical signs (akinesia, rigidity, resting tremor, flexed posture), and these signs are reversed by L-dopa or bromocriptine. The loss of pigmented nerve cells in the substantia nigra and the low levels of HVA in lumbar CSF correspond to the major pathological and biochemical changes found in idiopathic parkinsonism (1, 2).

In the present study we have shown that a similar neurological disorder can be produced in the rhesus monkey. Akinesia, rigidity, a flexed posture, and a postural tremor that can be reversed by the administration of L-dopa are evident after several intravenous doses of NMPTP. The signs induced by NMPTP in the monkey are directly comparable and similar to those produced in man (Table 2).

Table 2. Comparison of the major clinical signs of the NMPTP-induced neurological disorder in man and the monkey

<table>
<thead>
<tr>
<th>Man</th>
<th>Monkey</th>
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<tbody>
<tr>
<td>Akinesia</td>
<td>Akinesia</td>
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<tr>
<td>Rigidity</td>
<td>Rigidity</td>
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<tr>
<td>Resting tremor</td>
<td>Postural tremor</td>
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<tr>
<td>Flexed posture</td>
<td>Flexed posture</td>
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<tr>
<td>Eyelid closure</td>
<td>Eyelid closure</td>
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<tr>
<td>Difficulty swallowing or drooling</td>
<td>Difficulty swallowing (drooling)</td>
</tr>
<tr>
<td>Difficulty with speech or mutism</td>
<td>Decreased vocalization</td>
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</table>

NMPTP acutely affects brain dopamine, norepinephrine, and serotonin systems and produces decreases in the levels of their metabolites in brain and CSF. An initial decrease in the release of dopamine is shown by the rapid fall in the ventricular CSF concentration of HVA and decreased tissue levels of HVA accompanied by an increased dopamine content in the striatum. The accumulation of dopamine observed in the axons above the substantia nigra suggests that NMPTP may damage or destroy the terminal varicose fibers in the caudate–putamen. The axonal transport of dopamine is subsequently retarded and dopamine accumulates within the axonal processes.

Over a longer period, NMPTP appears to selectively and irreversibly damage neurons in the pars compacta of the substantia nigra (corresponding to areas A8 and A9 of the rat brain), leading to chronic cell degeneration and ultimately to severe nerve cell loss and a marked reduction in the dopamine content of the striatum. Dopamine terminals in the nucleus accumbens and olfactory tubercle originating from other dopaminergic neurons in the ventral midbrain (corresponding to area A10 of the rat brain) appear normal on examination by histofluorescence methods. Clearly, these two dopaminergic neuronal systems are differentially sensitive to the neurotoxic agent.

Although diminished biogenic amine release was evident in the lowered levels of HVA and MHPG in the ventricular CSF one day after the first dose of NMPTP, the monkeys failed to exhibit motor abnormalities until several doses of the drug had been administered. This is consistent with data showing that considerable reduction of dopamine and its metabolites may occur without the development of clinical evidence of disordered function, presumably because of adequate compensatory activity in the surviving neurons. Enhanced turnover of dopamine in the residual neurons presumably is reflected by the reversal of the HVA-to-dopamine ratios 27 days after drug administration.

Aging results in a diminution in viable neurons, including those of the dopaminergic systems. It has been suggested that the age-related changes in dopaminergic systems could be an etiological factor in the development of idiopathic parkinsonism or depression, the incidence of which increase with age (18). There is a possibility, therefore, that humans who are exposed to doses of NMPTP that produce permanent but subclinical damage to the nigrostriatal system may be susceptible to the development of parkinsonism at an unusually early age.

We have shown that NMPTP is the neurotoxic agent responsible for drug-induced parkinsonism in humans by reproducing the neurological syndrome and pathological changes in the rhesus monkey. A toxin causing a syndrome in animals similar to idiopathic parkinsonism had not been demonstrated previously, to our knowledge.